SCHOOL OF AVIATION MEDICINE
U.S. NAVAL AIR STATION
PENSACOLA FLORIDA

A STUDY OF THE PHYSIOLOGICAL CHANGES WHICH OCCUR DURING ACCLIMATIZATION TO HIGH ALTITUDE

AN INACTIVE FORM OF HEMOGLOBIN FORMED DURING ACCLIMATIZATION.

Research Project X-720(Av-376-s) Report No. 5

31 September 1946

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Research Report

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SUMMARY AND CONCLUSIONS

A form of hemoglobin which apparently is not active in the transport of oxygen has been found in the blood of four men acclimatizing to high altitude. This pigment resembles other inactive pigments previously described by Barkan, Ammundsen, Roughton and others.

In amount the pigment varied from 0.5 to 3.0 vols. percent during rest and was always less during exercise, as compared to the average value of 0.45 vols. percent found in seventeen determinations on eight individuals at sea level and during short periods of anoxia. No consistent relationship was found between the amount of pigment and blood lactic acid, sugar, pH_s, or the degree of anoxia present. In the acclimatizing subjects there was no consistent relationship of inactive pigment to ascorbic acid intake, but three normal subjects demonstrated an increase in inactive pigment during and for a short time after a few days of elimination of ascorbic acid from their diets.

The inactive pigment is very labile, being readily activated by short periods of moderate exercise, and does not appear to be methemoglobin as determined by a simplified photoelectric colorimeter (12).

In the course of a study of acclimatization to high altitude (1) it was noted that a small but significant portion of the blood pigment in four subjects did not readily combine with carbon monoxide or oxygen. This led to further study, the results of which strongly support the findings of other investigators that an "inactive" form of hemoglobin may occur in human blood. This preliminary report deals with the investigation of this "inactive pigment" during rest and during work under conditions of chronic anoxia.

Barkan (2, 3) in 1925 described a "pseudo-hemoglobin" in normal human blood which he believed to contain trivalent iron and to be inactive in the transport of oxygen within the body. His findings were strengthened by the work of Taylor and Coryell (4) who consistently noted the presence in normal human and bovine blood of hemoglobin-like substances which combined with oxygen or carbon monoxide only after the blood had been completely reduced by treatment with hydro-sulfite. They found the iron in this substance to be in the ferric form and pointed out that the oxygen or carbon monoxide capacity of blood does not give an accurate measure of the hemoglobin content, if appreciable amounts of the "inactive pigment" are present.

Similar findings were reported by Ammundsen (5) who found that in 40% of blood samples taken from 53 normal and 35 diseased persons there was present an amount of inactive pigment greater than 0.5 vols. percent which she considered to be the limit of error of her gasometric techniques. Roughton, Darling and Root (6) confirmed Ammundsen's findings and agreed with previous workers in considering that the pigment was not methemoglobin. This curious form of hemoglobin may explain the finding reported by Brooks (7) of as much as 10% non carbon monoxide combining pigment in the bloods of control animals which she attributed to an abnormal condition of the animals. The suggestion by Roughton, Darling, and Root (6) that the inactive pigment might increase during anoxia prompted the study of this pigment in the four men who were acclimatizing to high altitude.

METHODS

Detailed descriptions of the experimental conditions, the analytical techniques, and the complete blood determinations are given elsewhere (1, 8).

The amount of inactive pigment was determined by the difference between the carbon monoxide capacity of blood before and after treatment with sodium hydro-sulfite as follows: Arterial blood was drawn into an iced syringe containing heparin-fluoride solution and brought immediately to sea level for analysis. Within seven to ten minutes after drawing, two ml. of blood were exposed to eight ml. of 85% carbon monoxide in a ten ml. syringe which was rotated at room temperature for three minutes. The carbon monoxide was expelled, fresh gas introduced, and the rotation continued for an additional three minutes. This double exposure to carbon monoxide insured against incomplete saturation of the hemoglobin. All gas was then expelled and the syringe plugged to prevent further change in carbon monoxide content until the blood was analyzed by the Roughton Scholander technique (9). Oxygen and carbon monoxide contents of the blood were determined on another sample of the blood as drawn by the combined method of Roughton and Scholander (9), and the oxygen capacity of the blood was calculated by subtracting the CO content from the CO capacity.

For the determination of total pigment a 0.5 ml. sample of blood was treated with an equal amount of sodium hydrosulfite and saturated with carbon monoxide as described above. The difference between the CO capacity of the hydro-sulfite treated blood and that of untreated blood represents the amount of inactive pigment present.

These determinations, as well as those of blood sugar, lactic acid, and pH_s were made during rest and during a standard work test, and all bloods were handled identically. Because ascorbic acid has been reported to reduce other pigments such as methemoglobin (10, 11), the daily intake of ascorbic acid was measured by a dietician.

RESULTS

The CO capacities of both treated and untreated blood, and by their difference, the amount of inactive pigment present are shown in Table 1, which also contains the values found on the same bloods for lactic acid, blood sugar and pH_s. In Fig. 1 are shown the values for active, inactive, and total pigment during rest and work in chronological order, which is approximately the order of increasing altitude. No correlation between ascorbic acid intake and inactive pigment was found, and these data are

not included. Neither blood nor urinary levels of ascorbic acid were measured.

One determination of inactive pigment, made on Morris a month after return to sea level showed 0.4 vols. percent.

A few additional studies were made of normal resting subjects at sea level. Seventeen measurements on eight individuals gave an average value of 0.45 vols. percent. Three of these individuals voluntarily eliminated ascorbic acid from their diets, as far as this was possible, for three to five days. In every case the amount of inactive pigment increased and remained elevated for several days after normal diet was resumed. These fragmentary results are being studied more carefully and will not be further discussed at present.

DISCUSSION

The amount of inactive pigment found in the four acclimatizing subjects during rest was greater in nearly every case than can be explained by experimental error (0.5 vols. %). It was also greater than the average value found in seventeen measurements on 8 normal persons at sea level, and it was greater than the values reported by other investigators. During exercise the inactive pigment was usually less, and never more, than during rest, as can be seen from Figure 1.

Since the total pigment found during work was nearly identical with that found during rest (when both were determined), the two may reasonably be assumed to be the same in the other instances when only one was measured. Therefore, inasmuch as the amount of active pigment was less during rest than during work, it is highly probable that the inactive pigment was always greater during rest than during work. When the evidence is considered as a whole, it is clear that a pigment was present during rest which was not active in the transport of oxygen, but which was so labile that at least part of it was rendered active by seven minutes of work at the rate of 2,530 ft.lb./min. Whether activation occurred in vivo or in vitro we are unable to state with assurance, though the former appears the more probable.

The data in Table 1 indicate that neither lactic acid, blood sugar, nor pH_s, were directly related to the amount of inactive pigment present, despite the demonstration by Roughton (6), Barcroft (10) and Deeny (11) that any of these factors may effect the reduction of other inactive pigments, notably methemoglobin. In the four acclimatizing subjects there was no relationship between daily ascorbic acid intake (which was frequently very low) and the amount of inactive pigment (which was usually high) in comparison to the increased amounts (up to 1.5 vols.%) found in the three subjects who restricted their intake of ascorbic acid at sea level.

The average value of inactive pigment in normal subjects at sea level agrees well with the findings of other workers (2, 3, 5 6). When two men, shown to have 0.5 vols. percent or less of inactive pigment, were taken to 20,000 feet over a period of one to six hours, using both normal breathing and voluntary hyperventilation there was no significant change in the amount of inactive pigment despite striking changes in arterial pH_s, pCO_2 and $p[illegible]$.

These data indicate that during acclimatization (which is attended by a considerable increase in hemoglobin) the amount of inactive pigment increases above the level found in many normal subjects at sea level. No direct relationship can be traced between this inactive type of hemoglobin and arterial pH_s, pCO_2, and pO_2, blood sugar or lactic acid. Some influence developing during a brief period of exercise, greatly decreased this inactive pigment either in vivo or in vitro. Although no direct relation between ascorbic acid intake and inactive pigment could be found in the acclimatizing subjects, there is some evidence that such a relation exists in persons at sea level. This relation requires further elucidation.

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Date	Altitude 1,000 Ft.	REST Pigment-Vols.% Total	REST Pigment-Vols.% Active	REST Pigment-Vols.% Inactive	REST Lactic Acid mg. %	REST Glucose mg. %	REST Arterial pH_s	WORK Pigment-Vols.% Total	WORK Pigment-Vols.% Active	WORK Pigment-Vols.% Inactive	WORK Lactic Acid mg. %	WORK Glucose mg. %	WORK Arterial pH_s
[illegible]													
7/10	14	22.0	21.2	0.8	-	-	7.48	-	-	-	-	-	7.44
7/15	17	-	21.6	-	21	-	7.51	25.2	22.4	0.8	33	-	7.47
7/19	18.5	23.7	22.8	0.9	22	119	7.49	23.9	23.7	0.2	31	101	7.45
7/25	21	25.3	23.4	1.9	18	125	7.50	25.1	24.5	0.6	30	102	7.52
7/29	20	24.8	23.8	1.0	21	97	7.50	26.0	24.9	1.1	24	95	7.48
8/4	S.L.	21.7	21.3	0.4	23	-	7.37	22.2	21.9	0.3	22	-	7.39
MORRIS													
7/11	15	26.0	24.0	2.0	18	-	7.52	26.2	24.7	1.5	43	-	7.46
7/16	17.5	26.9	25.7	1.2	15	79	7.54	27.9	26.7	1.1	52	89	7.48
7/21	19	26.9	23.8	3.1	22	143	7.53	27.4	27.0	0.4	51	114	7.44
7/23	20	25.1	23.2	1.9	-	-	-	-	-	-	-	-	-
7/26	21.5	27.8	25.8	2.0	19	102	7.52	27.6	27.6	0.0	36	100	7.50
7/30	21	26.9	25.4	1.5	14	79	7.55	-	-	-	-	-	-
8/6	S.L.	-	18.6	-	-	-	7.45	-	18.5	-	-	-	7.42
HERTEL													
7/7	11	-	20.5	-	16	-	7.46	23.5	21.6	1.9	56	-	7.49
7/12	15.5	-	21.6	-	20	-	7.51	24.3	22.6	1.7	36	-	7.51
7/17	18	-	22.3	-	18	92	7.55	25.0	23.4	1.6	30	83	7.53
7/22	19.5	24.7	24.2	0.5	13	94	7.52	-	25.0	-	26	83	7.53
7/27	22	26.5	25.6	0.9	23	101	7.51	26.9	26.6	0.3	-	85	7.53
7/31	20	27.2	26.1	1.1	12	79	7.59	-	-	-	26	-	7.55
WILKINS													
7/8	12	-	21.9	-	18	-	7.47	24.0	22.8	1.2	30	-	7.44
7/13	16	-	23.7	-	15	-	7.48	25.1	24.6	0.5	32	-	7.49
7/18	18	-	23.1	-	17	99	7.50	24.3	24.4	0.0	32	88	7.47
7/23	20	27.9	25.8	2.1	12	93	7.49	-	26.2	-	30	94	7.49
7/25	21	27.9	26.3	1.6	-	-	-	-	-	-	-	-	-
7/27	22	28.3	26.9	1.4	-	-	-	-	-	-	-	-	-
7/28	20	28.0	27.7	0.3	11	120	-	-	-	-	-	-	-
8/1	20	28.5	27.2	1.3	14	100	7.52	29.0	28.3	0.7	-	-	7.53